A CONTRIBUTION TO THE KNOWLEDGE OF THE BIONOMICS OF SAND-FLIES

BY

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(*Received for publication* 17 *February*, 1922)

PLATE VIII

During the late campaign in Macedonia, members of the B.E.F., Salonika, suffered more or less continuously during the hot season from sand-fly bites and sand-fly fever. As seems to be always the case, the trouble was very definitely localised, the usual foci being either dilapidated villages in or near which units were stationed, or entrenched positions which had been long occupied. In the summer of 1918 there was a severe outbreak of the fever in and round Janes (where in one unit from mid-June to mid-August there was an incidence of 150 per cent. of the total strength), and I was instructed in the third week of June to investigate the conditions as regards sand-fly in the district. Owing to an attack of dysentery I was unable to proceed to Janes until the second week in August, when much valuable time had unavoidably been lost. The following pages reproduce almost verbatim a report on my investigations between August 12th and September 26th based on copious notes made on the spot. There have been incorporated also various observations made during 1917-1919 on *Phlebotomus* in Macedonia.

It may not be irrelevant to mention in passing that on my way to Salonika in July, 1917, I kept a constant lookout for *Phlebotomus* wherever the frequent stoppages of a troop train offered an opportunity of searching any likely spot. A species of the genus (probably *papatasii*) was first encountered at a small station near Beaune (Côte d'Or), and other examples were noted later at Orange (Vaucluse). At the time I was not prepared to find the genus so far north, but Major Joyeux has subsequently told me that during the war special search was made for *Phlebotomus* in France, with the result that *P. papatasii* was met with amongst other places near Beaune and as far north as Paris itself.

In Macedonia the species of sand-fly (*Phlebotomus*) investigated were three in number, viz., *P. papatasii*, Scop.; *P. minutus*, Rnd., and *P. perniciosus*, Newst., the first named occurring, at Janes, in by far the greatest numbers. Owing to the difficulty of discriminating between the females of these insects no attempt was made to determine specifically all the material collected, but in some hundreds of cases in which identification was effected *P. minutus* formed about 1 per cent. and *P. perniciosus* 2 to 3 per cent. of the total. Probably one is safe in saying that 95 per cent. of the sand-flies observed belonged to *P. papatasii*. Melanic forms of all three species were observed and in long series of *papatasii* many colour variations were noted, but the proportion of these to the total was not worked out. Really dark examples were, however, uncommon. From various sources it appears that by the date on which observations were commenced sand-flies were much less common than earlier in the season (June). Nevertheless, up to September 10th they were numerous wherever sought for, and in certain localities very abundant. Between the 10th and the 14th of the month there was a great falling off in the numbers found daily, but even so a good many could be taken up to the end of the month, and these later captures were still persistent in their attacks.

In connection with the association together and seasonal prevalence of these sand-flies, it is interesting to note that in 1917 (August to September) I did not certainly meet with *P. minutus*, while *P. papatasii* and *P. perniciosus* (though one or other occurred in numbers at Kalamaria, Salonika, Karasouli, Lahana, Nigoslav) were never taken together. In the present season, 1918 up to June 24th, only *perniciosus* occurred sparingly at Kalamaria. Again between July 24th and August 10th *perniciosus* (with one or two *minutus* latterly) was so abundant in the same place that in a single evening (9 to 11 p.m.) two hundred could be taken at light in one latrine. *P. papatasii*, though carefully sought for, was not detected. Newstead, in describing *perniciosus*, records a similar experience in Malta. 'Two examples of *P. minutus* were found in association with this species, but strange as it may seem, not a single example of *P. papatasii* was either captured or seen on these occasions.'

In 1917 sand-flies (*P. perniciosus*) were still common at Karasouli in the third week of October.

As to the part played by these three species respectively in carrying sand-fly fever in Macedonia, little evidence is to hand. Assuming that all are potential vectors, it is by no means certain that each carries the fever in proportion to its numbers. In any case *P. minutus* seems hardly likely to be of much importance in this rôle. As regards *papatasii* and *perniciosus*, the former is in my experience not only more active but more voracious and incessant in its attacks. In three localities where sand-fly fever was reported (Karasouli, 1917; Salonika, Janes, 1918) it was present in numbers, and in two instances was the only sand-fly taken. My present impression is that *perniciosus* is if anything a less efficient vector than *papatasii*, but the point is one which can be settled only by direct experiment.

The work done at Janes has included the following:—(*a*) A search for the early stages of sand-flies *in situ*. (*b*) A study of the habitats and habits of the imago. (*c*) Breeding and rearing of the flies. (*d*) Preventive measures.

A. SEARCH FOR THE EARLY STAGES

During the whole of my stay at Janes search has been made more or less continuously for ova, larvae, or pupae of sand-flies. In the first three weeks practically nothing else was attempted, but the result of protracted examination has been negative. Search has been made (1) in the soil itself to a depth of 6 inches, particularly near any indication of moisture; (2) along the sides of earth cracks and fissures so far as they could be followed; (3) beneath loose stones and in the superficial layer of cracked rock at or near outcrops; (4) between sandbags and loose earthy rubbish thrown up in erecting tents, marquees, etc.; (5) between mud bricks or masonry in the lower tiers of buildings; (6) in the dampest looking recesses of dug-outs and among débris beneath hay stacks.

This failure to find any early stages is parallel to the experience of many other investigators, and does not necessarily imply that search was made in wrong directions. Subsequent experience of ova and larvae from captive females indicated that the earth, etc., previously examined was much too dry, and that larvae, if they were to be found, should have occurred at greater depths. It is possible, however, that larvae are absent or much less plentiful while the adults are numerous. Newstead, who worked in Malta in July, August and the first week of September was under the impression that larvae would be more numerous in autumn about a week after the adult had disappeared. As has already been stated, sand-flies began to decline in numbers at Janes between the 10th and 14th of September, and it was on the 20th that I found newly-hatched larvae for the first time in my breeding-boxes (see below, App. III, No. 1).

B. HABITATS AND HABITS OF FLIES

Habitats. (1) In mid-August, and for some time afterwards, sand-flies occurred practically everywhere in and near the C.C.S. They were more numerous, however, in tents and wards. In dug-outs they were comparatively scarce, which was at first rather surprising since at the same time one had a report that up the line the flies swarmed in dug-outs. The explanation appeared to be simply that the flies gathered as near as possible to their hosts. The hospital dug-outs were unoccupied, while the others were regularly used. In the same way where two wards, one occupied the other empty, adjoined, the former yielded many more flies than the latter. Again in a ward which, as sometimes happened, had only a corner bed occupied, the flies were most numerous in that corner. In large marquees or wards the distribution of the flies varied from day to day and with the hour of the day. After a windy night few could be found, and those that occurred were in sheltered corners. They were most abundant after still, damp nights. In the morning one found them mainly beneath the flaps running round the top of the sides of the tent, and the flies so taken showed a large percentage of newly-fed, gorged females. Later in the day, from roughly 11 or 12 a.m. to about 2 p.m., the flies became temporarily scarcer, while during the afternoon they were more frequently seen in numbers on the lower half of the side, males being numerous and often predominating. Pairs *in cop.* were common in the morning. Where the sides of marquees, closed during the night, were left standing by day more flies were found. Invariably, too, where the sides were rolled up into a corner, flies were found within the roll. When the ground at the corner was cracked, flies were observed emerging from the earth and entering the folds of the roll, so that even after clearing such a roll in the forenoon one might later in the day take many flies from the same place.

(2) Earth cracks, in fact, proved to be effective day-shelters for *Phlebotomus*. The most important proved to be those occurring on the exposed surface of the soil where it had been dug out to make a level floor for tentage. The presence of flies in the cracks was easily demonstrated by blowing tobacco smoke or by squirting into the lower portion a little paraffin or even plain water. Within a tent twelve to fifteen flies might thus be driven from a crack less than a foot long and a few inches deep (August); when so expelled the flies generally came back after a short flight, and they had to be pretty thoroughly disturbed before they would travel any distance. Sometimes when driven from one crack they merely hopped along the cut surface and entered another.

(3) Sandbags employed to raise the level of the sides of tents, etc., are also a fertile source of trouble. The flies rest not only in the crevices between the sacks, but also enter the loose earth inside through the interstices of the coarse sacking. Where dug-outs are near wards, and their entrance is reinforced with sand bags, many flies will be found. In the tent which I occupied during my stay at Janes were several courses of sandbags. The following experiment was many times verified. When one opened up the tent and allowed strong light to fall so that one side of the person was illuminated and the other side in the shade, as one stood with the hand extended towards the sandbags, then though no flies had previously been noticed, bites began to be received in a very short time. After one or two such trials it was possible to see the sand-flies emerging from their retreat to the attack, which was always made on the shaded side. Besides the situation indicated, sand-flies shelter by day in hanging clothes, cupboards, blankets, beneath pillows, etc.

Habits. In studying the habits of *Phlebotomus*, much difficulty was at first experienced in keeping the flies alive. In test-tubes many died within two days, and few survived the third day. Not even when the air was kept humid could oviposition be induced.

In the end, suitable apparatus for handling the flies was improvised, and a list is appended.

Various devices for keeping the flies alive were tried during the first four weeks, and ultimately earthenware pots for single or smaller lots and cages for larger numbers proved most useful (see Appendix I). In the earthen pots—which were first suggested to me by Lieut.-Col. C. M. Wenyon—single specimens were easily kept and handled as follows. The fly was caught in a test-tube over the end of which a piece of cotton was loosely tied so as to form a small bag. The test-tube was then inserted through the sleeve of the cover. A little water was next poured into the pot and the cover tied on, the test-tube being fixed at any desired height above the water by a piece of string passed round the sleeve (fig. 4). Flies so kept showed an interesting periodic movement, staying all day in the cool, humid air below and appearing at the top of the tube during darkness. They were generally restless—particularly *papatasii*—about dusk. In cages provided with a tray filled with moist earth, stones, etc., a similar movement was observable, the flies hiding in crannies by day and emerging by night. A considerable proportion, however, remained by day crowded together in corners of the cage. They were frequently observed drinking from the wet earth.

In collecting sand-flies a pot was taken and covered and the flies introduced into the sleeve from the test-tube in which they had been caught. The sleeve was then tied and the pot stood in a bowl of water (fig. 5). To feed them it was necessary, first, to turn the flies loose for a time into a fly-proof box fitted with a sleeve to admit the arm (fig. 3). It was noticeable that flies kept in solitary confinement would seldom feed if the tubes were merely inverted over the arm.

In pots, unfed, a variable proportion of the flies—up to 50 per cent.—lived a week, 2 to 3 per cent. lived nine days, one ♀ (out of a batch of six hundred) lived exceptionally thirteen days. No ♂ was observed to live more than eight days under these conditions. All the females that lived nine days or over were examined as to the condition of their ovaries, and were found to be spent or practically so. In no instance could they be induced to feed in this state, though offered repeated opportunities—in the case of the last extending over the eleventh, twelfth and thirteenth days.

(1) *Biting, etc.* No males were detected in this act. The large number of this sex found with the females (as compared with *Culicidae*, where often the total catch from a ward will not include a single ♂) seems to be due to the fact that mating takes place after the female has had a blood feed. In all the couples examined the female had recently gorged herself. While feeding *Phlebotomus* is easily disturbed, the slightest movement of the skin being sufficient to put the fly to flight. It is thus difficult to study the process of biting in detail. The insect settles, pitches forward slightly, thrusting the somewhat stout rostrum downwards while the palpi (maxillary) diverge a little. I have not seen more than about one-third of the rostrum enter the skin, and the labium does not buckle up as in *Anopheles* when engaged in the same act. Only the labella are flattened out above and behind the piercing part of the associated organs. The wings are meanwhile poised ready for instant flight. Blood can be seen in the sucking stomach within sixty seconds. A full feed on an empty stomach occupies from four to four and a half minutes, at the end of which the fly suddenly withdraws the rostrum and makes off. For about forty-eight hours after a meal blood can be seen in the 'sucking stomach' over-lying the mid-gut (whose contents are brown or blackish) on the left side, and the whole gut may be cleared in five days, but the process generally takes longer. The females will feed at two-day intervals (possibly at shorter periods), taking less copious feeds, but attempts to find how many feeds could intervene before the completion of oviposition were abandoned. The digested blood is passed out ultimately in the form of small, dark, sticky drops; one or two specimens when captured had the stomach gorged with pale fluid and the gut hardly darker.

(2) *Movements.* In cracks and on rough earth *Phlebotomus* ordinarily proceeds by short runs varied by jumping to one side or another; on smooth walls or fabric more by jumping and short flights. Confined in a test-tube the males commonly mount in seeking an outlet. Females, if recently fed, on the other hand settle downwards, but after the gut has cleared and the eggs laid they behave much as do the males. When first introduced into a cage or confined space sand-flies make determined efforts to escape. They pass through astonishingly small openings, using the proboscis

apparently as a lever and emerging sometimes with a considerable proportion of the scales and hairs missing.

(3) *Drinking.* As *Phlebotomus* runs over the surface of a piece of earth it may sometimes be observed to plunge the rostrum into the earth. If there is moisture present the fly may remain for some time still. To observe what was happening, specimens were isolated in plaster of paris cells and watched under a Zeiss binocular. After they had been imprisoned for twelve hours the block was placed in clear water, which at once mounted to the floor and sides of the chamber containing the flies. Practically all the insects commenced to drink, some of them running about excitedly for an instant before settling. As was noted before, the tip of the rostrum (labrum, epipharynx and hypopharynx, etc.) was thrust distinctly into the plaster, and the labellae, flattened out, closely appressed to the porous surface. The short superiorly ensheathing maxillae generally moved backwards and forwards for a short time in front of the clypeus, and the maxillary palpi—rather widely divergent, nearly at right angles to one another—moved tremulously. But during drinking the mouth parts were still. Swelling of the abdomen could be traced as the drink proceeded. Flies, both sexes, were found to drink readily in this way about twice daily. They were tried with drops of fresh human blood on paper slipped into the cell, but without result. This can hardly be regarded as conclusive, as the blood dried rapidly. Defribrinated diluted blood was next allowed to soak into the cell containing flies, but did not prove specially attractive. The flies appeared to be able to extract only diluted serum—the corpuscular débris being retained by the plaster. In the same plaster cells cut deeper *Anopheles* was also induced to drink water.

C. BREEDING AND REARING OF FLIES

(1) *Oviposition.* While the conditions in pots standing in water were congenial to the adult flies, there was apparently insufficient moisture within the pot to induce egg laying. Nor did females kept in a tube over moist paper oviposit. Complete success was, however, attained by the following methods. A thick 'tray' was partly filled with fragments of earth over which was lightly

sprinkled some crushed faeces of lizard, rabbit or man. The tray was then soaked in water and placed in a pot into which water had been poured to a depth of a quarter of an inch (fig. 5). The flies were then put in through the sleeve as usual, and the pot stood in water. After an interval the pot was opened; on washing out with as small a quantity of water as possible (20 c.c. was sufficient as a rule) a number of eggs were got from the sides. They were collected as follows. The liquid was centrifuged and the clear part used to rinse the pot again. This was done three times. Finally the sediment containing the ova was pipetted off and the ova themselves separated out under the microscope. The percentage of fertile eggs was extremely high, and their viability was not appreciably affected by their being for some time in water.

Besides the ova taken from the sides of the pot many were to be noted on the tray or among the earth, etc. To recover eggs so laid was an extremely tedious process. Some were picked up with a brush by direct observation, others recovered after carefully washing the contents of the trays. An improvement in gathering ova from the sides of the pot was effected by lining the inside with a single piece of cotton pressed down to fit exactly. The pot was then loaded as before. The eggs (which are at first pale and then darker like those of mosquitoes) showed up well against the cotton which was cut into strips and so passed below the microscope. A further advantage was that the cloth showed definitely the average position of the eggs laid on the sides of the pot to be along a band about three-quarters of an inch above the water surface (fig. 5, *o*.) This seems to indicate that there is a fairly precise optimum as regards moisture for oviposition. Eggs on the sides of the pot occurred singly, but the majority were found below the earth close to one another in bunches. The females had crawled into the smallest crevice possible and appeared to have projected the eggs still further. Beside all the larger collections of eggs in these cracks the mother could be found within two millimetres. She lay, as a rule, flattened between the surface of the tray and the overlying earth, with sometimes one or two eggs between the extremity of the abdomen and the rest of her laying. Sometimes an egg was seen attached to the terminal bristles of the genital appendages, and once or twice females had died with the egg between these appendages or blocking the genital atrium. With a little care, it was easy to clear the earth from round each dead female and count the eggs laid. The larger batches contained from twenty-seven to thirty-four eggs. Sand-flies are not prolific insects, and the total ovarian content in *P. papatasii* runs from forty to fifty eggs. Assuming that these females had previously deposited one or two isolated ova on the sides of the pot, the proportion of eggs actually laid to the total possible is a high one. In some hundreds so secured there were practically none that failed to hatch. This high fertility and the large number of eggs laid may reasonably be held to indicate that natural conditions for oviposition had been secured.

Newstead, who in 1910 watched egg laying of *P. papatasii* in Malta, states (*a*) that the egg is projected some distance from the abdomen of the female; (*b*) that the process is so exhausting that the female may die after it; (*c*) that most of the eggs were laid *below* the moist blotting paper supplied to induce oviposition. With these notes the foregoing observations are quite in harmony, and it seems probable that the violent ejection of the ova serves to insert them into crannies where the abdomen of the mother cannot penetrate. Newstead saw the ova thrown about three times the length of the female abdomen, and this again agrees closely with the distance noted by myself.

In cages where the earth, etc., was placed on a shallow tray no excessive moisture ran up the sides (the water being completely absorbed), with the result that eggs occurred, so far as could be seen, only in pockets with dead females close by.

An attempt to determine whether the sand-flies in ovipositing showed any preference for one kind of faeces was inconclusive, owing to the development of microfungi, whose ramified mycelia enmeshed the eggs so that a complete count was impossible. The fungus growth was slight on lizard faeces either by themselves or mixed with earth, but very abundant on human faeces. So far as one could judge, however, plain earth or earth and lizard faeces held more eggs after some hundreds of females had been allowed to oviposit on plaster trays giving a choice of several kinds of larval food.

No female was noted to oviposit till four or five days after capture, and the eggs did not hatch till at least nine days more. The incubation period probably varies seasonally and specifically.

Ova belonging to at least two species were secured. Of these, one, the larger, was by its size, abundance, texture and resulting larva plainly *P. papatasii*. The other (probably *P. perniciosus*) was shorter and a little stouter. Apart from size, the two could easily be told towards the end of the period of incubation by two dark parallel lines which appeared shining through the shell laterally in each. In *papatasii* the lines at the caudal end of the ovum were rather broader and tapered towards the head. They could also be traced round the head and back towards the tail for a short distance. In the second species the calibre of the lines altered little between tail and head, and they extended nearly half-way back towards the tail on the other side. After hatching, these lines could be recognised as the caudal bristles, which are relatively short in *papatasii*. All through the first instar there is a kink in the caudal bristles, indefinite and near the extremity in *papatasii*, and much more decided and further back in the second species. The kink is, of course, at the point where the bristle is bent within the egg.

For the health and ultimate hatching of the egg a considerable degree of moisture is necessary—rather less, however, than is required to induce oviposition. Excess of moisture for a limited time has no effect on the viability of the eggs, for they may remain immersed a day or over and yet hatch. Eggs resting on earth surrounded by a thin film of water for three to four days also hatched. Drying, on the other hand, even a short time is fatal. A batch of eggs exposed in the shade overnight shrivelled before 11 a.m. next day, while in the daytime a few hours brought about the same result. (Time, 20th-25th September.)

(2) *Larvae.* When the egg is about to hatch the caudal bristles may be seen to move. There is a rippling movement also of the body from the tail towards the head on which, high up, almost vertical in position, is the well-developed dark egg tooth. Dehiscence of the shell is affected by a cut extending sometimes to half the length ventrally (?) and backwards for a short distance dorsally (?). The eyeless and legless maggot emerges slowly, and is at first entirely pale save for the egg tooth and bristles. The head, however, darkens in a few hours. In emerging the larva seems to be coming from two valves, but in some cases the line of

dehiscence is cut laterally as well as dorso-ventrally so that a relatively large oval piece of chitin falls from one side of the shell. Excessive moisture retards the process of hatching, always a slow one, and when the surface on which the egg rests has a thin covering film of water the larva may be found barely clear of the shell twenty-four hours after hatching began.

The newly hatched larva is sluggish, and, indeed, during the whole of this instar little activity is shown. It lies either flat on the supporting surface with the caudal bristles extended in the same line, or resting on the ventral caudal third to one-half of the abdomen, with the rest of the body raised and the last segment with its bristles slightly upturned. In this pose the larva is U-shaped. In a modification of this attitude the head is again thrown forward and the whole creature in profile S-shaped. The larva's progress is undulating. The head is first raised and the anterior segments stretched forward. The mandibles now press (or grip ?) firmly some inequality of the surface, and with this purchase the body is dragged slowly forward. Folds in the skin, and possibly the peculiar hairs of the body, aid in the process.

The tiny larvae begin to feed almost as soon as the head has darkened, *i.e.*, the mandibles are hardened. In feeding on lizard faeces they select the rough portion consisting of chitinous fragments mixed with partially digested fibre, and reject the more homogeneous limy part. Three individuals watched settled down respectively to the mandible of an ant, the head capsule of some small hymenopteron and the leg of a beetle, and ate the half-digested muscle fibre inside these structures. In the same way they enter and feed on the dead bodies of the parent flies. Older specimens of the first instar will also attack and devour larvae immediately after hatching.

Fungus was so troublesome (being rightly or wrongly blamed for the loss of many young larvae) that some substitute food was sought. Finely ground mixed blood and earth has proved satisfactory for this purpose. It is spread in lines on shallow plaster trays with the eggs near, and the whole covered with rough pieces of earth. The young larvae feed readily and appear to thrive, and no fungus has as yet developed.

Larvae in the first instar can survive excess of moisture for some time. They sink readily in water, and are not sustained for any length of time by the surface film. When dried after lying twenty-four hours on wet soil they are lethargic but soon recover, yet like ova they are extremely sensitive to thorough drying and shrivel if exposed a few hours in the shade.

The first ovum to hatch did so on 20th September, and the first example in the second instar was seen on 26th September. In this stage the egg tooth has, of course, gone; the caudal bristles have increased from two to four, with a dark chitinous saddle connecting them. There is also a considerable increase in size.

In India, according to Howlett, there are separate broods of *P. papatasii* in August and September, while the wintering brood begins in late October or early November. At Janes I have seen no trace of the September brood, and the slow growth of these larvae hatched from late September eggs suggests that in Macedonia there may be one less brood than in India, and that the wintering brood commences here at least a month earlier.

Subsequent experience confirmed my expectation that the September hatched brood would hibernate. It was impossible to give much time to observing the larvae during the last week of September and the first fortnight of October, owing first to the congestion of the C.C.S. after the push, and later to the severe epidemic of influenza. But about the middle of October a considerable number of larvae, mostly in the second instar, were successfully taken to the Base and installed in the laboratory at 52 General Hospital. They were by this time very sluggish, and, in spite of being kept in moistened earth in a room which was heated at least during the daytime, latterly ceased to feed, while some died. One lot which had been left as it came from Janes was allowed inadvertently to dry up completely. On examining this tray in the third week of November (by which time it must have been quite dry for a month) I could find no larvae moving on the surface, but on breaking up the earth as a precautionary measure before throwing out the contents of the tray, I found several larvae in little pockets or blisters in the earth. They lay quite straight out but appeared stout and contracted, the integument wrinkled slightly towards head and tail, the gut empty or practically so. The only movement exhibited was a slight twitching from side to side of the extremities when the animal was gently touched. By bringing larvae in this condition into moist, warm surroundings it was possible to revive them so that they recommenced to feed. Unfortunately the few experimented with were accidently destroyed.

The bulk of my larvae had not revived naturally at the end of March, 1919, and the attempt to bring them to London failed. While it is not suggested here that *Phlebotomus* hibernates naturally under such conditions as have just been described (the opposite, indeed, is much more likely), it is evident that these larvae, once the critical stages of hatching and the first instar have been overcome, have unexpected powers of resisting dessication which must be of considerable help to the species in its natural breeding haunts in cracks, etc., where the conditions as regards moisture are variable.

D. PREVENTIVE MEASURES

(1) *Nets*. During my stay I used the sand-fly net issued to the troops with satisfactory results. With a flash-light at night one could see the flies settling on the net, yet none were noted to pass through. Once or twice sand flies (up to four) were found within the net in the morning, but these, I believe, came not from the outside but from blankets or below pillows, having got there during the process of bed-making. They also find harbour inside sleeping bags, and all bedding should be thoroughly shaken or beaten just before the net is adjusted for the night.

In this connection, however, it is worth recording that of thirty to forty mixed sand-flies placed in a small bag of the material from which the nets are made and hung up loosely, all escaped within half an hour. The flies behaved as they do when confined in a cage, pushing in all directions to find an outlet. I have found in the same way that a certain number of mosquitoes in a brood covered over with ordinary netting will manage to struggle through. The species in which this was noted were *Culex pipiens*, *Anopheles palestinensis* and *A. bifurcatus*. In spite of this the net in use appears to be efficient, though possibly where *P. minutus* preponderates a finer material might be necessary.

(2) *Repellents.* (*a*) Ordinary *Paraffin* is, if liberally applied, effective in keeping off the flies. It might be used as a stop-gap. M.T.C. drivers reported that they had employed it with good results, but I do not regard its regular use as advisable, and made no tests personally of the time for which an application remains effective. (*b*) *Mosquito Pomade* and (*c*) *Paraquit* were both tried. The former was issued to various members of the personnel of the C.C.S., who reported favourably on its use. Of the two, Paraquit is perhaps more pleasant to use. Well rubbed in, I found it effective for about three hours when one was sitting still or not moving actively. I found it sufficient to rub the Paraquit into the back of the hand, wrist and halfway up the arm towards the elbow, the neck, ears and round the scalp, but not into the hair. For the bites themselves I found appreciable relief by dabbing on a little rectified spirit with cotton wool.

(3) *General.* The breeding-sites of *Phlebotomus* hitherto recorded have been varied. Larvae have been found in soil along the sides of the embedded portion of the lower corners of old masonry; in rubbish in cellars, in earth cracks, etc.—the common feature in each case being darkness, moisture, and the presence of organic débris. While it is possible that at 31 C.C.S. the flies came in part from farm buildings near by, I think it most probable that the main breeding-places were in or round the hospital itself, in the cracks where the adults were themselves found. There was nothing in the distribution of these adults to suggest that their breeding-sites were other than generally distributed. Where there were more flies there were more men sleeping. In such circumstances prophylactic measures are difficult to carry out, and the only remedies available are probably palliative rather than radical.

The following suggestions are given for the treatment of tents and marquees. The floor should be levelled and cracks filled up with a mixture of *cresol* and *sand* or *sawdust*. *Clay* is unsuitable, as it is apt to crack in turn (this was repeatedly noted) and the most minute fissure will harbour many flies. The floor should then be liberally watered with a strong solution of cresol, and if possible covered with a ground sheet. Periodically, according to the severity of the plague, the tent should be closed and sprayed with a solution of formalin or fumigated with cresol. 1 per cent.

formalin has been recommended for spraying, but I believe this is too weak. In corrugated iron huts a 1 per cent. mixture of cresol in paraffin emulsion might usefully replace formalin for spraying. Around the tent all cracks (to a breadth of two feet from the tent) to be filled up and the soil to be sprinkled to the same width with cresol.

In latrines where crude cresol was experimentally sprinkled round the drums, sand-flies were reduced in number for two to three days, and ceased to bite on the exposed legs though bites were still received on the neck. A similar reduction was noted in a store where cresol was regularly sprinkled.

As far as possible, shallow soil with frequent outcrops of loose friable rock should be avoided in choosing a camp site. Loose soil removed in pitching tents should not be allowed to remain in camp. Building up with sandbags round tents, etc., is to be deprecated. Spilling of water or any liquid containing organic matter should be carefully avoided. The appearance of cracks in the soil should be watched for and counteracted by filling up when possible and spraying with cresol when the cracks are more extensive. From ten to fourteen days after the disappearance of the successive waves of sand-flies that occur from June to September a thorough sprinkling of cresol over suspected breeding-sites is to be recommended.

I have to thank heartily Lieut.-Col. Ievers, D.S.O., O.C., the 31st C.C.S., for his kindness in affording facilities for work during my stay at Janes, and very specially Lieut.-Col. C. M. Wenyon, C.M.G., who first suggested the investigation.

In conclusion, I should like to record my indebtedness to Captain Beer and Corporal Gibson, R.E. (143 Field Company) for carrying out my design for the breeding-cage (fig. 1), and to Captain Morrell, Dental Officer to the C.C.S., who supplied the plaster trays and cells.

APPENDIX I

Apparatus used in Studying the Life History of *Phlebotomus* spp.

Cages may be made to suit requirements. (*a*) The following dimensions housed comfortably 600 sand-flies, 13 in. broad × 14 in. high × 6 in. deep, made of 1 in. wood, back of tin driven into the wood and nailed, on inside faced with cotton glued down and whitewashed. All joints glued and rabbeted. One or two panels of cotton fixed closely by strips of wood to afford ventilation. Tin tray to hold water in bottom. Flies admitted by tightly-corked circular opening at side. Front glazed to depth of about 10 in.—the glass set in putty. Narrow panel door below glass 12 in. × 2 in., made fly-proof by cloth edging. All round the door the joint is rabbeted and felt lined. The door may be

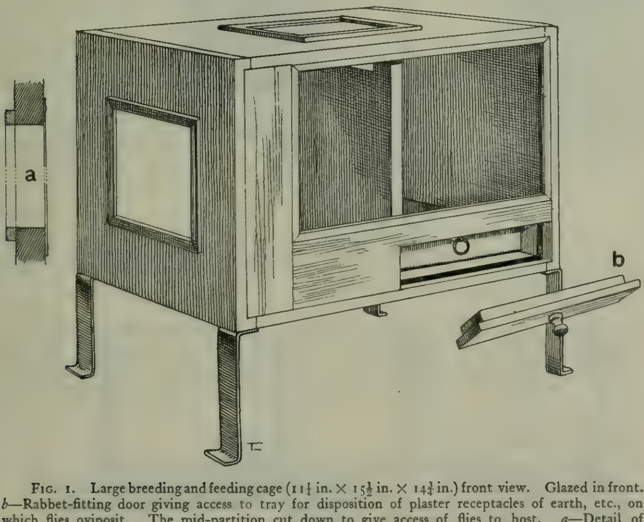


Fig. 1. Large breeding and feeding cage ($11\frac{1}{4}$ in. × $15\frac{1}{2}$ in. × $14\frac{3}{4}$ in.) front view. Glazed in front. *b*—Rabbet-fitting door giving access to tray for disposition of plaster receptacles of earth, etc., on which flies oviposit. The mid-partition cut down to give access of flies to host. *a*—Detail of ventilation showing the inner screen of stout wire-gauze and the outer cotton cloth.

secured by a clip. The middle surface of the rabbet pressing against the felt makes the joint fly-proof. (*b*) The above cage (no fig. given) is suitable when ova and larvae only are wanted. When the adult flies have to be fed for some time in numbers a more elaborate cage is required (figs. 1 and 2). It consists of two chambers. In the right-hand one the flies oviposit on material in tray. In the left chamber a rabbit or other small mammal can be accommodated for a time, but should not remain continuously with the flies. The animal sits on a wire grating and its urine and faeces are caught in a tray. Ventilation at top and side. In the latter case the ventilator is double—cotton outside and strong wire-gauze towards the rabbit. Inside the door and fitted to the sides is a large sleeve through which the animal is introduced to the cage. In the middle the sleeve is held closed by an elastic belt to prevent the egress of the flies. The rabbit is introduced by forcing its head through the confined middle of the sleeve and sliding back the band over its body. The flies are put in as in cage (*a*) and gain access to their victim over and below the mid-partition, which is cut back for this purpose.

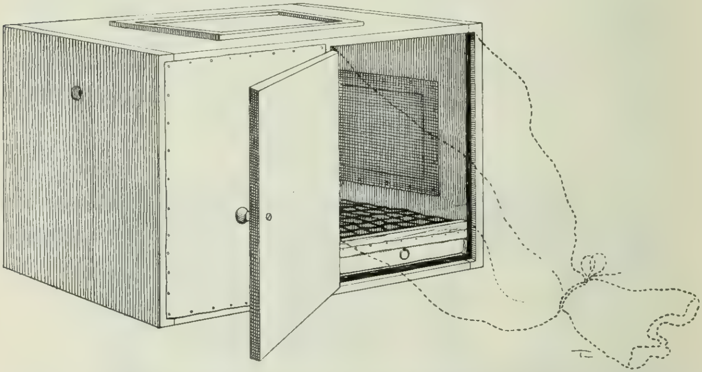


FIG. 2. The same from behind, showing the grid on which the rabbit is accommodated. Below, next to the door, is a wooden bar with a felt extension to make the faeces and urine tray fly-proof. The space above the grid is sleeved.

FEEDING CAGE. A quinine tin (10½ in. × 4½ in. × 6⅝ in.) with strong lever lid (fig. 3) was used for this purpose; the lid was cut out leaving only the rim and the reflexed edge. A row of holes about ⅛ in. apart was drilled along the latter and a sleeve sewn tightly on; a side was next cut away to ¼ in. from edge

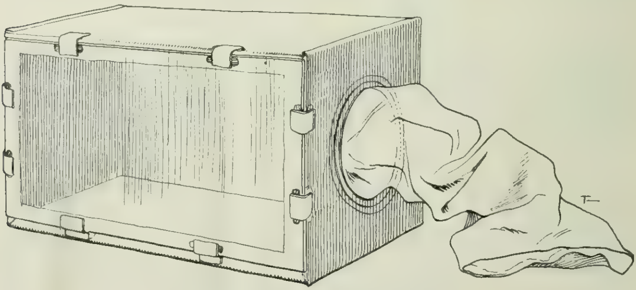


FIG. 3. Feeding cage with detachable sleeve.

and eight small clamps were soldered on, two at each side. The front was now glazed and puttied, and in the space between glass and clamps wedges of softish wood were fixed to prevent displacement of the glass. The inside of the cage was whitewashed. Flies kept in pots were released from their test-tube into the cage, fed, and easily recaptured.

The fact that no exact information was available in Macedonia as to the methods and apparatus used by previous workers was at first a considerable handicap. While it was easy to improvise ways of handling the flies in small numbers, many attempts were made before a satisfactory cage for dealing with large numbers of *Phlebotomus* was evolved. From the first cage made, 250-300 flies escaped in twelve hours, and only the closest search revealed the tiny escape hole. Some of the first boxes were constructed with grooved joints **U**, and the flies actually squeezed themselves round the **U** and so escaped. In other cases the flies disappeared gradually from the cage, but were not observed to escape. On taking the box to pieces they were found dead, packed solidly in the joints. Afterwards glued joints were tried with success. The superfluous glue must be carefully scraped off and the joint dusted e.g. with dry plaster of Paris to prevent the flies sticking. Glass jars are not suitable for housing *Phlebotomus* for any time, as the insects are liable to get stuck down by their wings.

In loading a pot with flies, the covers and tray are arranged as in fig. 5. The test-tube is then introduced into the sleeve perpendicularly, when the ♀♀ will generally drop down without trouble. The tube still in position is now laid

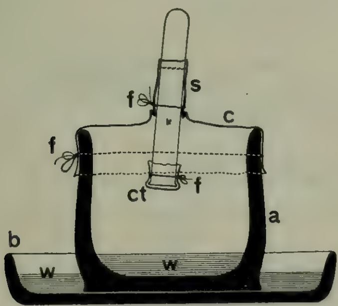


Fig. 4. Method of confining single flies required for observation. *a*—pot; *b*—outer tray; *w*—water; *c*—outer cover of pot with *s*—sleeve; *ct*—cover of test-tube; *f*—fastenings.

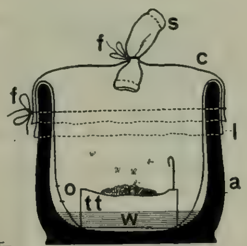


Fig. 5. *a*, *w*, *c*, *s* and *f* as in fig. 4; *l*—cotton lining of pot; *tt*—thick tray with faeces, etc.; *o*—approximate position of the zone on which most of the eggs not laid on the bait occurred.

horizontally for a moment and rapidly raised again to the first position. The ♂♂ come out, fly upwards, and are caught by the inner projection of the sleeve when the tube may be withdrawn.

The pot cover in fig. 5 is shown loose to prevent confusion with the inner lining, but in practice it must be tightly stretched and firmly secured, otherwise the flies squeeze down as far as they can get between the cover and the lining and are apt to be killed when the pot is lifted.

CELLS and TRAYS of PLASTER OF PARIS (fig. 6, *a-d*).

CELLS. These were cast a little wider at the base than at the top. In shape square. Measurements at base $1\frac{3}{4}$ in., at top $1\frac{1}{2}$ in., depth $\frac{3}{4}$in. The corners and angles were bevelled off. On the upper surface a square ($\frac{1}{2}$ in. side) was cut to a depth of $\frac{3}{16}$ in., over this was laid a No. 1 $\frac{3}{4}$ in. cover-glass, and the surface gently scraped away round the central hole till the cover-glass slid easily backwards and forwards over the cavity.

TRAYS. (*a*) *Thin* for cages, oblong, 3 in. $\times$ $2\frac{1}{2}$ in. $\times$ $\frac{3}{8}$ in., with a central hollow about $1\frac{3}{4}$ in. in diameter sunk to about half the depth of the tray. (*b*) *Thick* for pots, circular, 2 in. diameter and $\frac{3}{4}$ in.—$\frac{7}{8}$ in. deep. At one side a small length of wire, bent to form a handle, was let into the mould to facilitate lifting the tray from the pot. The central hollow of these thick trays was made by a 2 in. watch glass.

EARTHENWARE POTS of local manufacture, rough unglazed, circular, $3\frac{3}{4}$ in.—4 in. in diameter and about the same height, with an inside depth of 3 in. ; average thickness of walls $\frac{1}{4}$ in. (see fig. 7, *a*, *b*). Covers for the above (fig. 7, *c*) were made of light, but closely-woven cotton cloth. In the centre was sewn a short sleeve of the same material. The sleeve was about $1\frac{1}{2}$ in. broad by $2\frac{1}{2}$ in. long above. It is an improvement to have it project below about $\frac{1}{2}$ in. to prevent the ready egress of the flies after they have been introduced into the pot.

Wooden cages are loaded through the circular aperture at the side, which can be kept closed by a cork. Flies, if thirsty, generally go easily into observation cells, but sometimes ♂♂ can be induced to enter only by inverting the cell above the mouth of the tube and catching the ascending *Phlebotomus*.

The thin, flat trays are useful for isolating any given set of ova or larvae which it is desirable to watch. Their containers should have some water.

All cages and pots should be insulated (e.g., by water) to prevent depredations by ants, etc.

The sketches have been made by Mr. Terzi from apparatus brought back by the author.

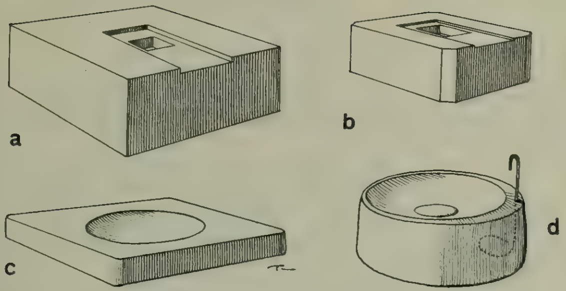


Fig. 6. *a*—large cell to demonstrate drinking habit of ***Phlebotomus***, *b*—smaller block with larger well in which isolated ova were kept and development from day to day noted; *d* and *c*—thick and thin trays.

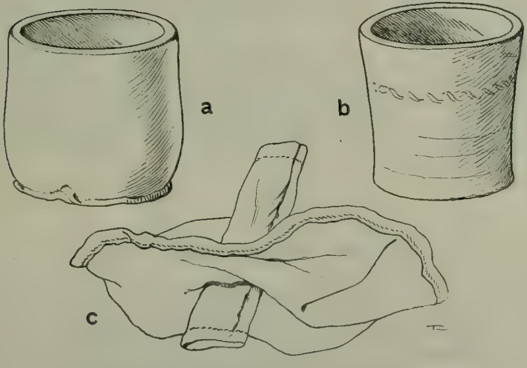


Fig. 7. *a* and *b*, Earthenware pots. *c*, Cover with sleeve.

APPENDIX II

Feeding of the Larvae of *Phlebotomus*

Faeces. Three kinds were used. To avoid any unpleasantness they were well dried and powdered before use, except in the case of the lizards' excreta, which were not offensive.

(*a*) *Lizards* (caught in some cases in cracks tenanted by sand-flies) were kept in a cage whose front and bottom were of coarse wire-gauze. The cage stood over a tray in which the faeces collected. The lizards were fed at first from fly-traps and on various insects specially caught for them. Latterly it was found that they would take grasshoppers readily, and as these *Orthoptera* made a more quickly satisfying meal and were easily secured, this became the staple ration for the lizards. The faeces were stored in test-tubes and did not generally develop fungus. Only the portion first passed, consisting of fragments of the exo-skeleton of insects to which some of the undigested internal parts still adhered, was of use. The more homogeneous urates—a limy-looking mass—were rejected.

(*b*) *Rabbit faeces*, well dried and crushed roughly.

(*c*) *Human faeces*, dried for twelve hours in oven and reduced to a very fine powder in a mortar.

Plain Earth. Dried at air temperature ; small stones removed ; powdered in mortar. Small larvae were observed to ingest this, doubtless for the sake of the associated bacteria, etc.

Earth and Blood. Earth prepared as above. Human blood haemolysed, but not defibrinated. The two were mixed to the consistency of a thin mud. Next dried in oven into a cake, which was reduced again to a powder and stored for use. This was a most excellent food for young larvae. They gathered about the lines where it was laid, and its passage into their gut could quickly be traced under the binocular.

NOTE ON TABLE (APPENDIX III)

From the middle of August to mid-September daily catches of *Phlebotomus* (up to 300 per day) were made at Janes. In all about 3,000 specimens were handled. Notes are given below on some of these lots so collected. Although I frequently watched *Phlebotomus* sucking blood, I do not think that a full feed was made on more than six or seven occasions, in all of which the times taken were very uniform, there having been not more than 15-20 seconds difference between any of them. A note on one such case is appended. No. 8.

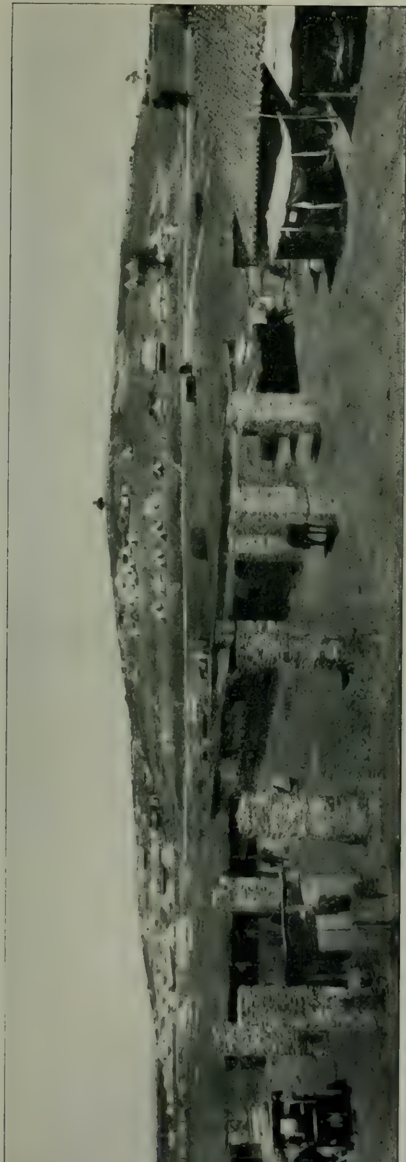
No. of experiment	No. of flies	Larval food	Results of experiment			Surviving unfed
1	50	4.ix.18 Confined over lizard faeces	12.ix.18 All dead save one pair: ♂ moribund—died in two hours; ♀ active, refused to feed, though given choice of two hosts, died in 12 hours; ova laid in pot	20.ix.18 Ova hatched	26.ix.18 Larva (1) noted in 2nd instar	♂ 8 days. ♀ 8½ days
2	70	6.ix.18 Lizard faeces	16.ix.18 All dead. Ova laid, numerous, several batches, up to 34. Much mildew in faeces	20.ix.18 12 ova placed on blood and earth, all hatched	21.x.18 Many larvae hatched. Two spp. present	
3	100	7.ix.18 Broken rabbit faeces and earth	16.ix.18 All dead save one ♀; she will not feed; ova, several, enmeshed in mildew	18.ix.18 ♀ dead	21.x.18 Many larvae hatched. Two spp. present	♀ 11 days
4	120	8.ix.18 Human faeces on earth lump	18.ix.18 All dead, not many eggs		21.x.18 Many larvae hatched. Two spp. present	
5	6-700	8-10.ix.18 Lizard, human, rabbit faeces in long tray	11.ix.18 Many dead	19.ix.18 None alive, though yesterday a ♀ was active; eggs found below broken pieces of earth	26.ix.18 Tiny larvae found completely enclosed in pockets formed by the breaking down of earth during moistening	♀ 8-10 days
6	100	Human faeces and earth	23.ix.18 All dead. Many eggs, but few on earth where mildew was dense; eggs in zone on cloth			
7	100	13.ix.18 Earth only	24.ix.18 Only one ♀ alive, will not feed; ova very numerous	26.ix.18 ♀ died, has not fed		♀ 13 days
8	1 ♀	28.ix.18 Specimen caught feeding. Gorged in 4 mins. 25 secs. Confined in test-tube in jar	30.ix.18 Sucking stomach half empty	1.x.18 Meal now visible only in mid-gut, which is about half-full and dark	7.x.18 Died	REMARKS.—This specimen lived an hour or two over 9 days after feeding. Showed the usual night and day movements. Deposited faeces freely. 29.ix.—5.x.18

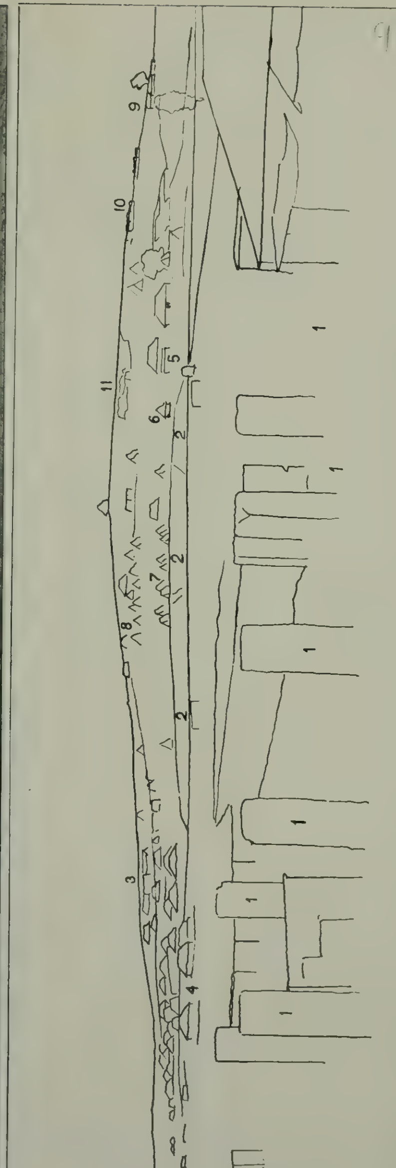
EXPLANATION OF PLATE VIII

General View of Janes—31st Casualty Clearing Station, September, 1918.

1. Courtyard, pillars, etc., of Janes Farm.
2. Low, much cracked escarpment at roadside.
3. Hospital,—Wards, Sisters, etc.
4. Stores.
5. Pathological laboratory, Workshop, and Sanitary Section.
6. Entomological laboratory.
7. Personnel of C.C.S.
8. Officers.
9. Isolation wards.
10. Isolation wards,—Offices.
11. Personnel (N.C.O.'s).

The original centre of distribution may have been—1, where the flies were always numerous in cracks in the lower courses of the masonry. They were to be found also by day along 2; and men in 7 suffered severely. The largest catches were made at 9 and 10.





J. W., Photo.

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